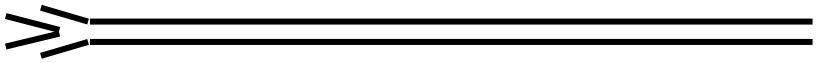
Chapter 7



Biological Methods

7.1 Introduction

Although the initial streamlining proposal pertains only to chemical analytical methods, EPA intends to expand method flexibility to include biological methods in the future. Biological methods include both the testing of an environmental sample for the presence of microbiological material (e.g., bacteria, protozoa, and viruses) and the use of biological organisms in tests for whole effluent toxicity (WET) of an environmental sample. EPA believes that flexibility in testing for biological material will be similar to the flexibility allowed in the modification to chemical analytical methods. Test procedures should be able to be modified when the modifications produce equivalent or superior results. EPA has protocols for some microbiological methods that are currently used in the alternate test procedure (ATP) program (EPA 1996b, 1996c). EPA is developing a protocol for approval of new and modified (alternate) WET methods that is based on the tiered validation structure provided by streamlining.

Biological methods are considered to be method-defined analytes. As discussed in Chapter 2, incorporating flexibility into method-defined analytes will likely require more rigorous control than modifications for specific chemical substances. EPA believes, however, that certain parts of the procedures can be modified without adversely affecting method performance. At present, this problem has not been sufficiently addressed to allow proposal of specific flexibility requirements in approved biological methods. Until EPA can clarify the extent of acceptable flexibility, requests for changes in biological methods will be reviewed and approved on an individual basis.

OW is working with EPA's Biological Advisory Committee (BAC) to identify appropriate applications of flexibility in WET test methods. As mentioned above, EPA also is developing a protocol for approval of new and modified (alternate) WET methods that includes procedures for external organizations to develop, validate, and submit WET methods or method modifications for EPA approval. This protocol will be distributed for comment after it is completed and has undergone internal EPA review.

EPA anticipates that requests for approval of new or modified (alternate) WET methods will focus on one of the following areas: organism; test duration; test procedures; reactor type (e.g., batch, flow through, or fill and draw); equipment, volume-to-organism ratio, or system monitoring. Factors that will be considered in reviewing submitted methods include: single- and multi-laboratory precision; the life- stage, sources, and quality of test organisms; the nature and control of test conditions; test data collection and reporting requirements; test acceptability criteria; endpoints; methods of data analysis; and test sensitivity.

7.2 New WET Methods

The following has been suggested as a definition for a new WET test method:

A WET test procedure will be considered a "new" procedure if it employs a "new" species or requires culture conditions, test conditions, endpoints, and/or methods of data analysis that are substantially different from those used for current Agency-approved species/methods.

7.3 Modified WET Methods

The following has been suggested as a definition for a modified (alternate) WET method:

A proposed test procedure will be considered a "modified" procedure if it involves only minor changes in established test conditions for an approved species/method, or if it employs a "new" species to be used as a substitute for a related, Agency-approved species, and if:

(1) The proposed test with the "new" species can be performed with essentially the same test conditions and methods of data analysis used for current Agency-approved species/methods (i.e., with only minor modifications in one or a few conditions), and
(2) The sensitivity of the proposed test species/method using an approved or "new" species is demonstrated to be equal to or greater than the sensitivity of current Agency-approved species/methods, using reference toxicants or effluents, or
(3) The proposed test results in a significant reduction in the cost or ease of performance of the test, without an unacceptable loss in sensitivity.

7.4 Validation Requirements

In keeping with method flexibility guidance, laboratories would be required to demonstrate that a modified (alternate) method produces results equivalent or superior to those produced by the EPA-approved reference method and would be required to demonstrate that new methods produce data that are acceptable for use in NPDES compliance monitoring. It has been suggested that this demonstration would consist of paired side-by-side tests with effluents and a range of reference toxicants (metal, organic, and salt).

It has been suggested that the following would suffice to document validation of a new or modified (alternate) WET method:

- **Summary of Method:** For modified methods, including a discussion of how the modified method differs from the 40 *CFR* part 136 method and the rationale for requesting the modification

- **Toxicity Test Procedure:** The method or modified portion of the method prepared in EPA standard format.

- **Data:** Data from paired side-by-side tests using both effluents and a range of reference toxicants (metal, organic, and salt).

- **References:** Including all sources of technical information used in developing the new method or method modification.